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Frequency of the K303R Mutation Stratified by Nodal Status

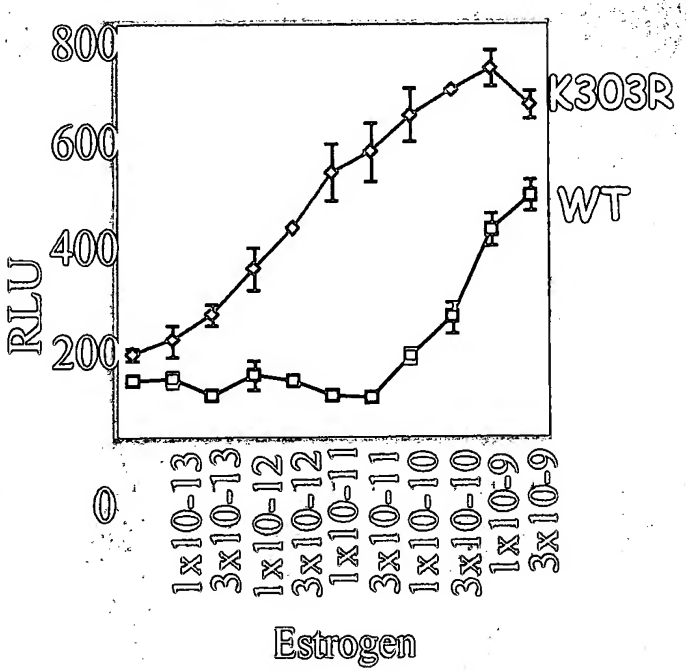
	WT ER	MUT ER
Axillary N-	16	10 (38%)
Axillary N+	4	23 (85%)

p=0.00062, Fisher's Exact Test

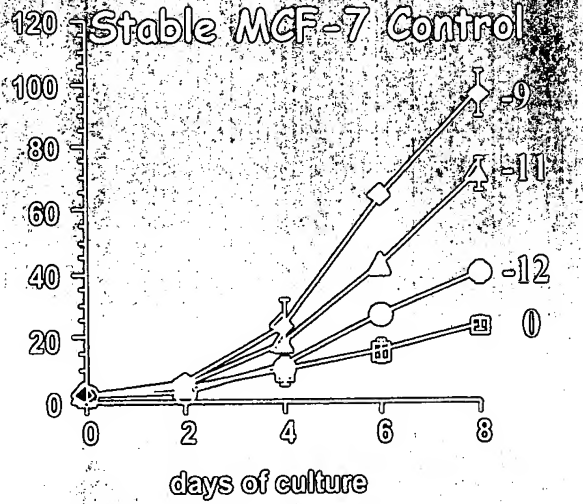
The Mutation is Associated with Advanced Breast Cancer

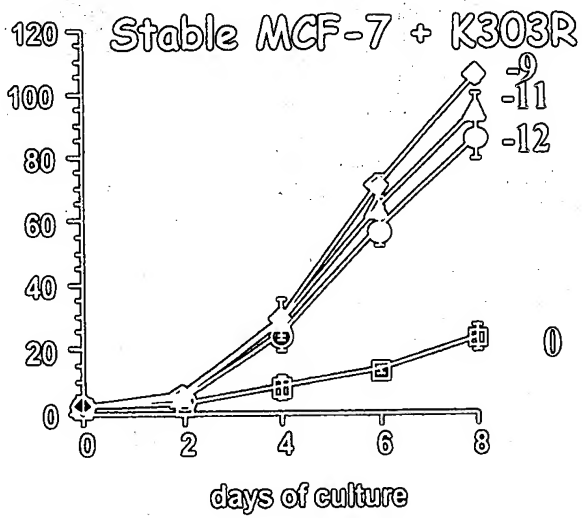
Hypothesis:
Proliferative Advantage is due to Hypersensitivity to Estrogen

Transactivation MDA-231

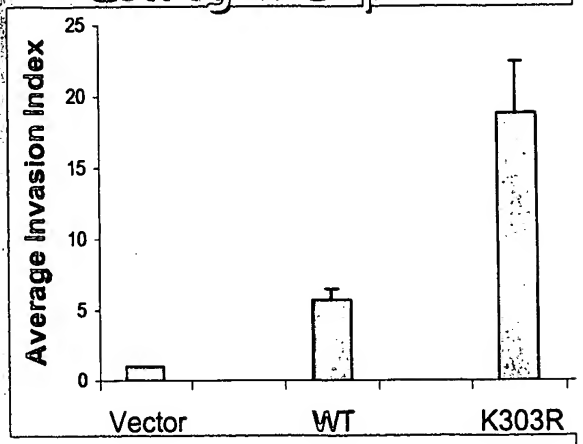


Growth Assay





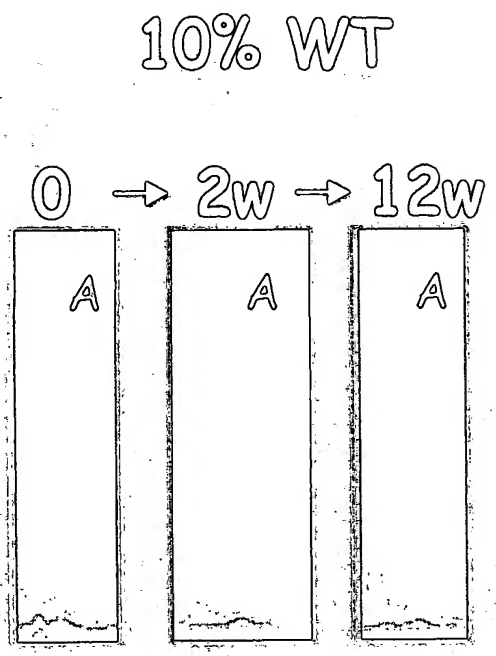
Matrigel Invasion Assay- Estrogen Depleted

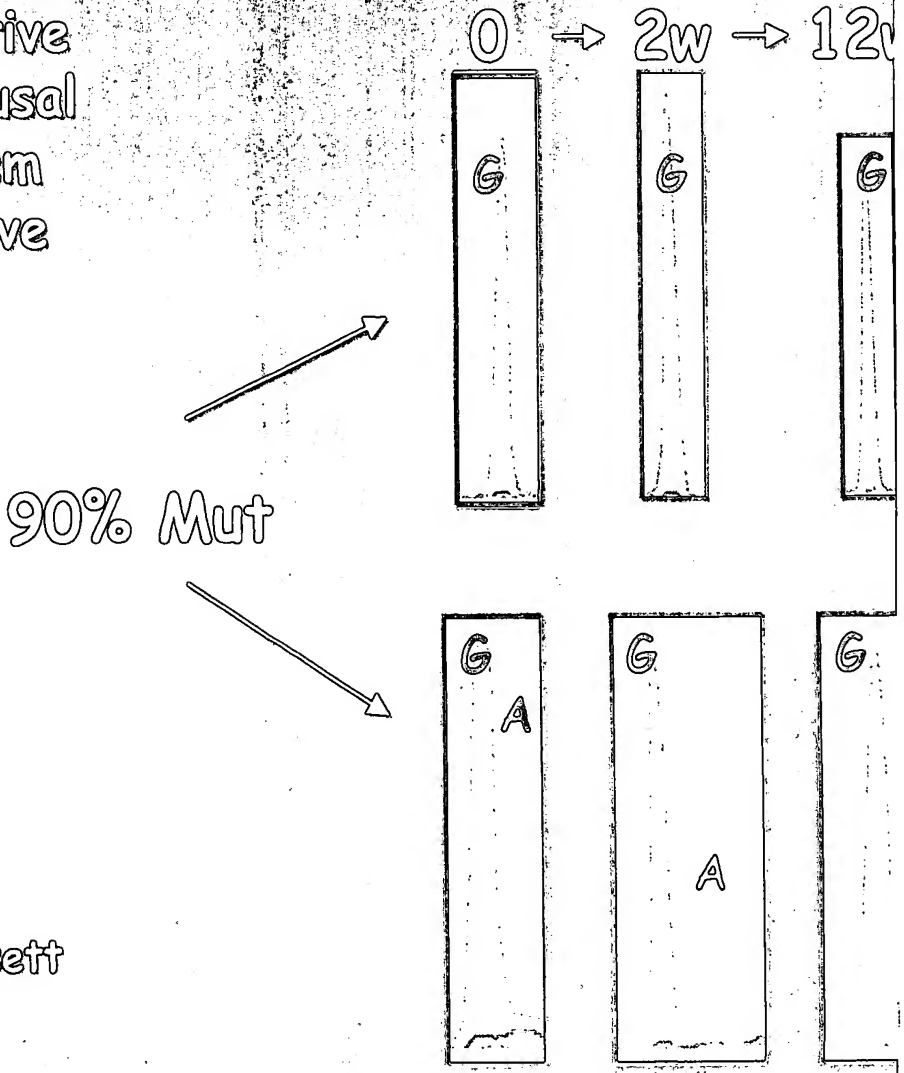


The K303R ERα Mutation is Frequently Present in Postmenopausal, ER-positive Patients

Neo-adjuvant study of 30 primary invasive breast cancers from UK**:

Node-negative
Postmenopausal
Size > 2 cm
ERα-positive

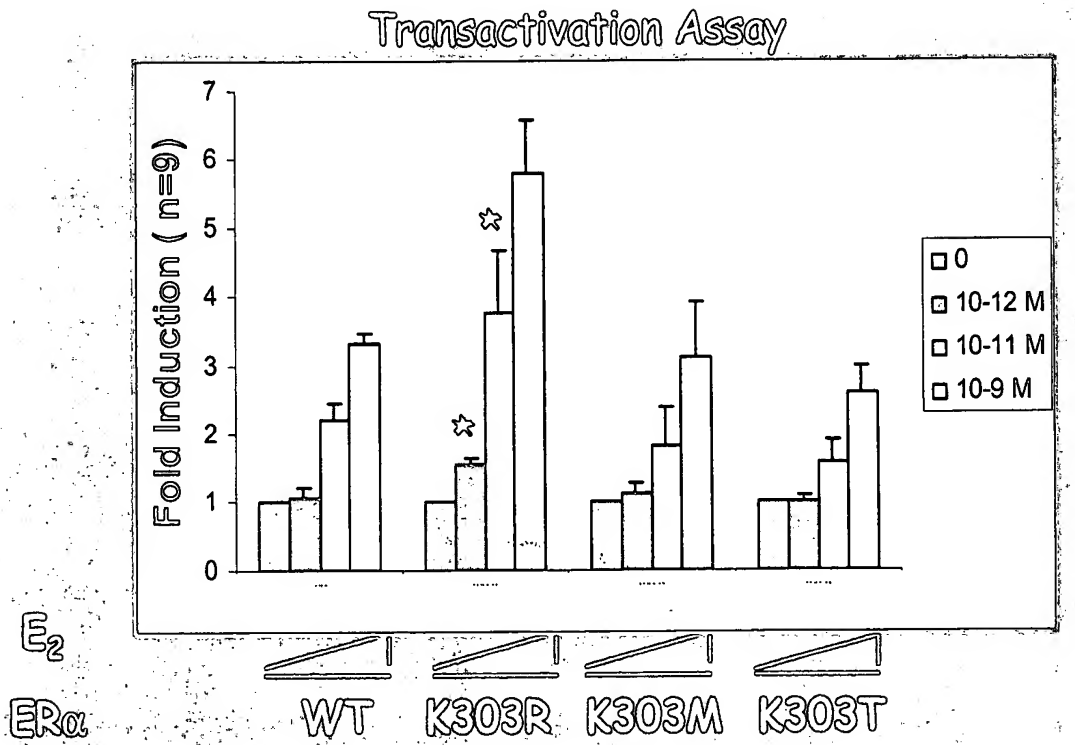




** M. Dowsett

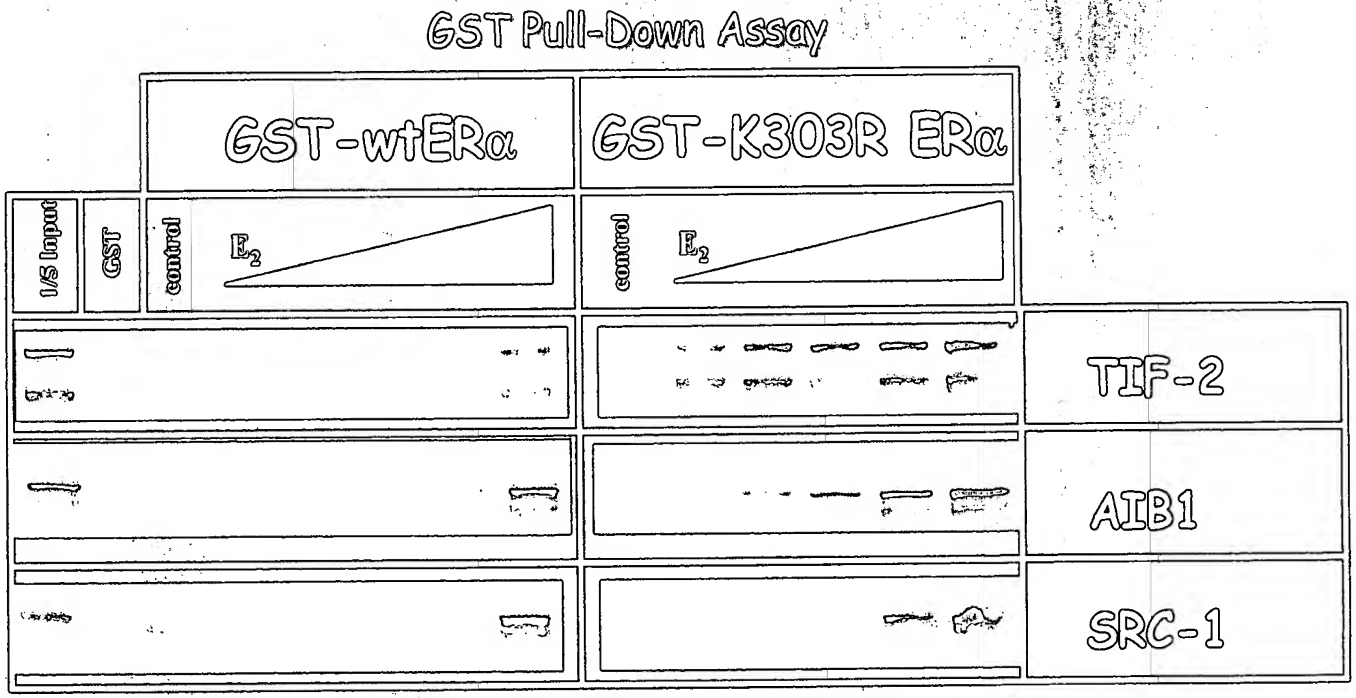
Why Find Only A to G Mutation in Tumors?

A to G = K303R
A to T = K303M
A to C = K303T



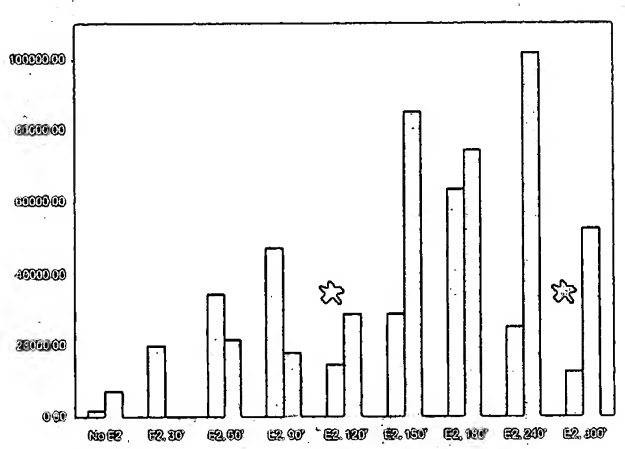
Only K303R is Hypersensitive

The ERα Mutation Exhibits Altered Binding to TIF-2 and AIB1 ER Co-activators

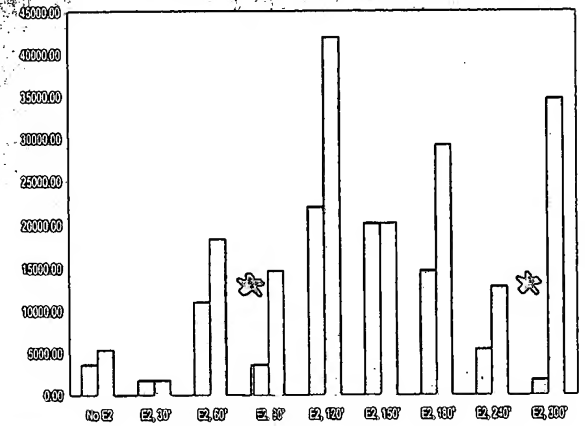


Altered Dynamics of K303R ERα on the pS2 Promoter

WT K303R

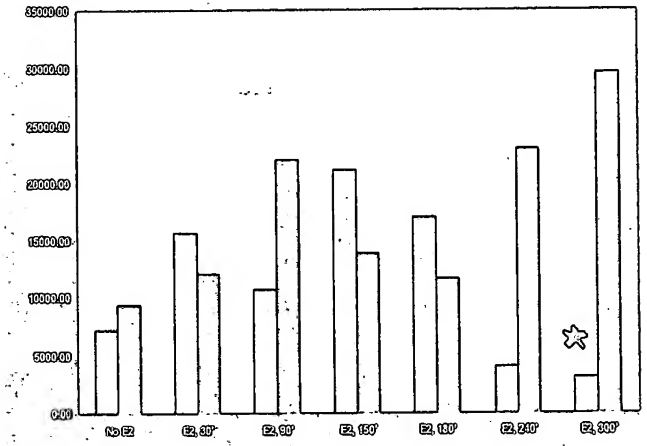


α ERα



α AIB

Mutant: increase in estrogen-induced occupancy on the promoter

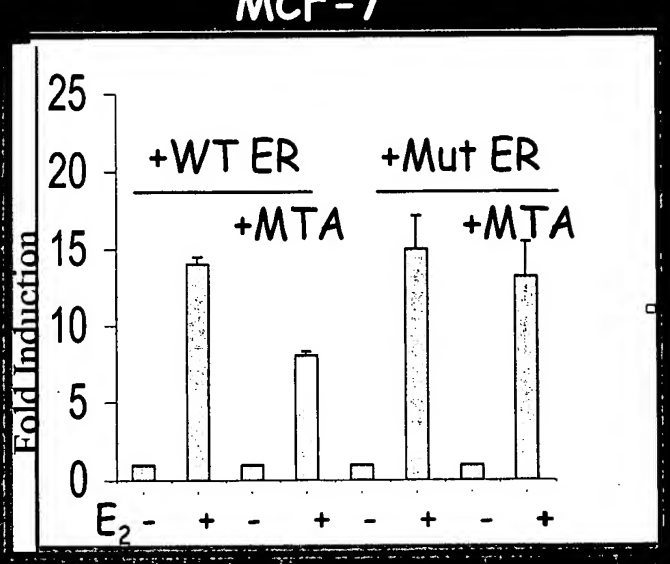


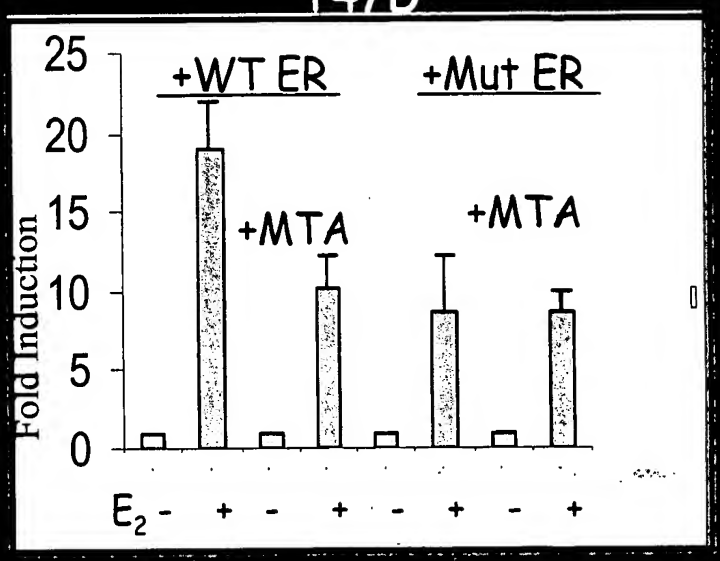
α p300

Mutant: p300 appears to be retained in the complex

The K303R ERα Mutant is Resistant to MTA2 Co-Repressor Inhibition Of ERα Activity

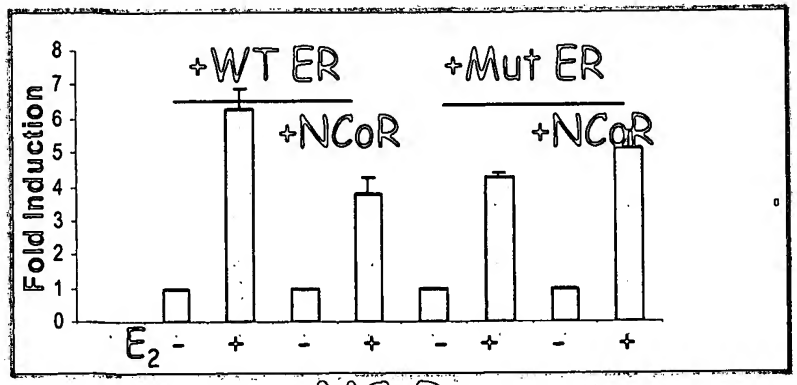
Transactivation Assays



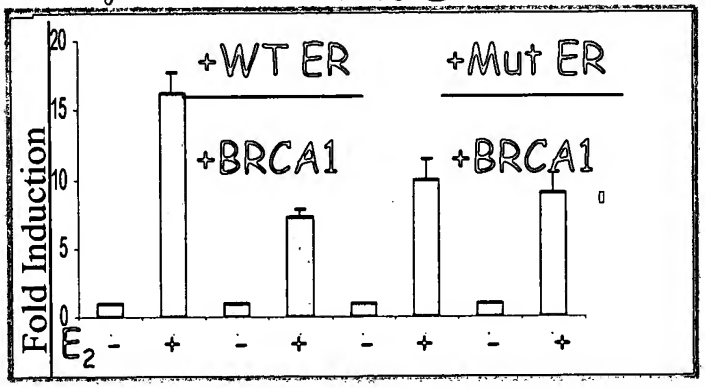


e K303R ER Mutant is Resistant to NCoR and BRCA1 Co-Repressor Inhibition of ERα Activity

HeLa Transactivation Assays T47D



+NCoR



+BRCA1

The K303 ERα Site is Acetylated by p300

3, GATA-1 motif

R X K K → Acetylated by HATs (p300 & P/CAF)

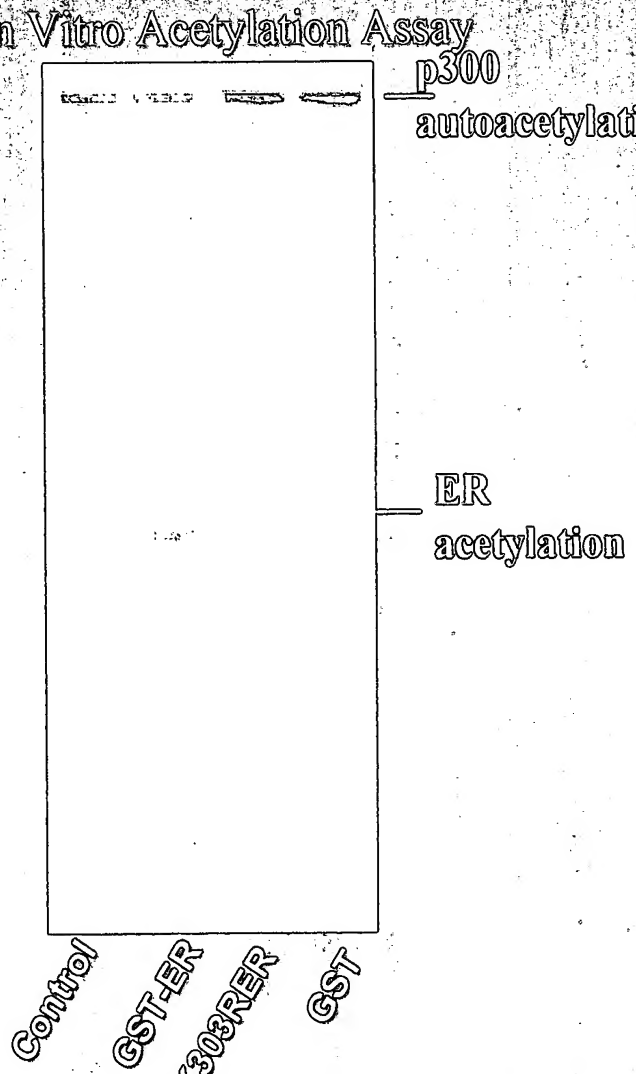
WT ER motif

R S K K → Acetylated by p300, not P/CAF

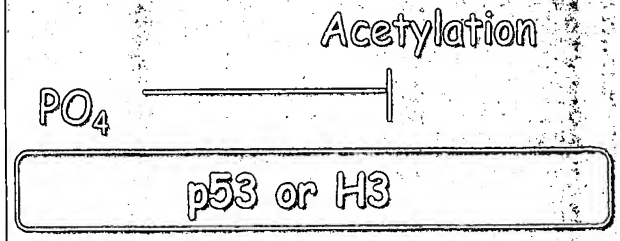
↓

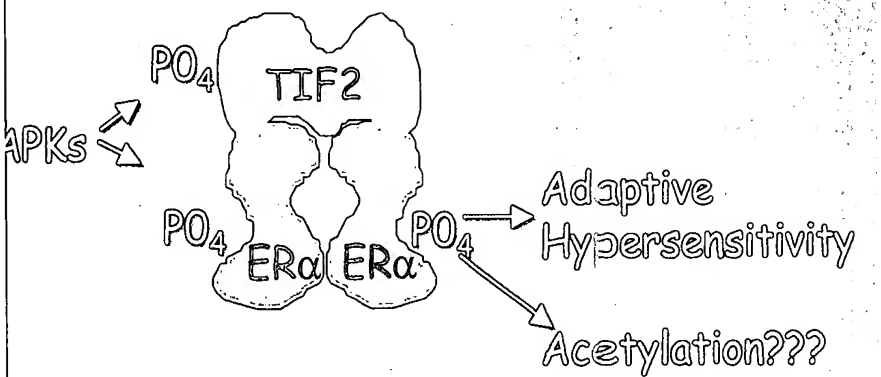
Mutant 303 R ⊣ Acetylation by p300 is blocked

Wang et al., JBC. 2001

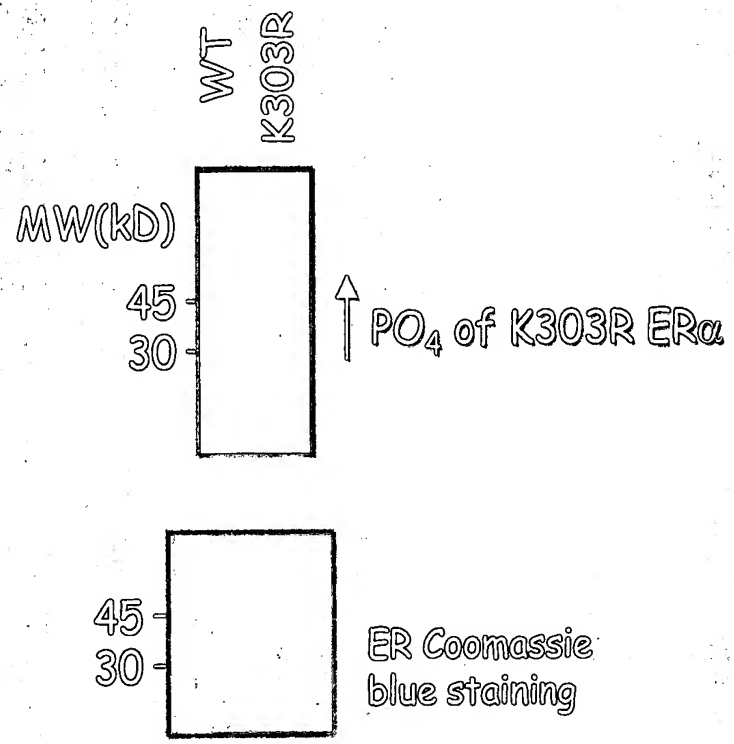


re Receptor Acetylation and Phosphorylation Coupled Events?





Total *In Vitro* Kinase Assay:
GST-ERs Hinge #253-310
+
MCF-7 Lysate
+
γP^{32}



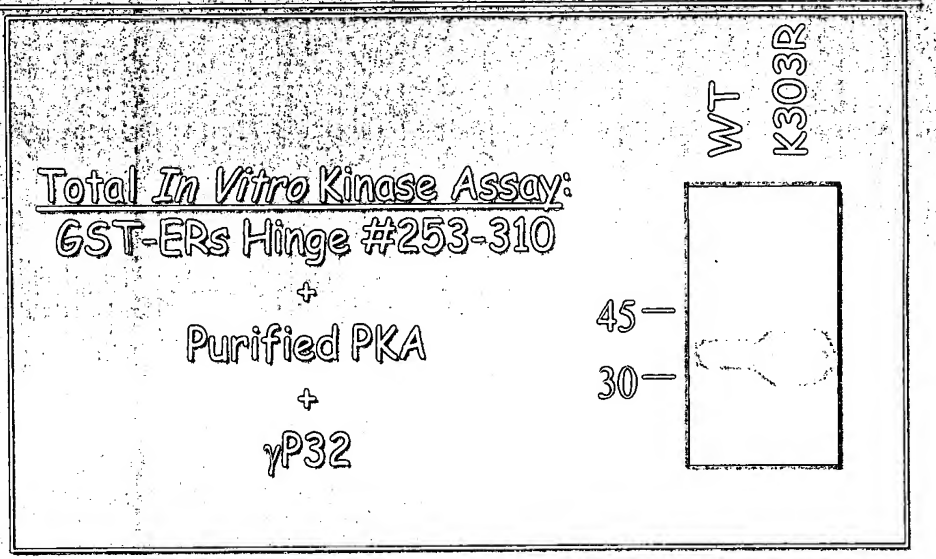
K303R ERα Mutation Generates an Efficient PKA & PAK1 Phosphorylation Site

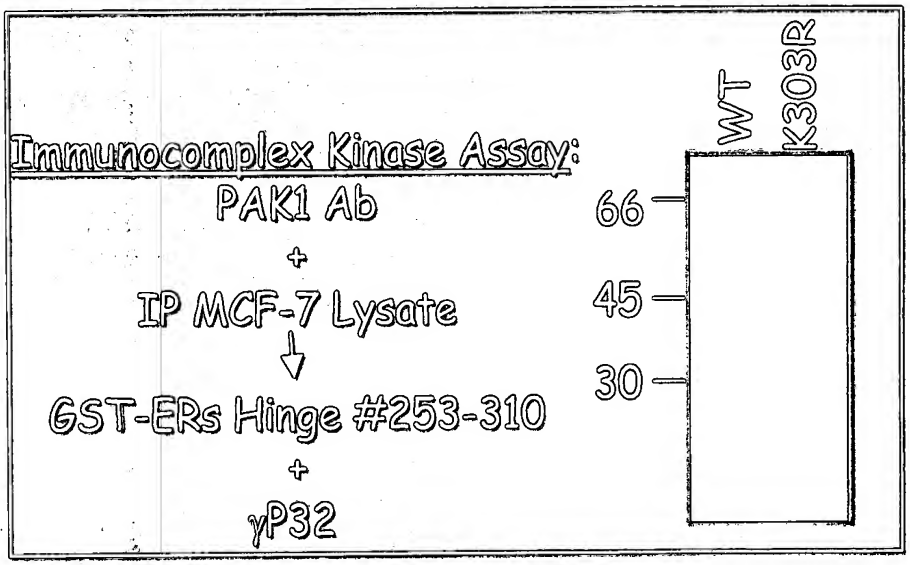
Kinase	Substrate Specificity	
PKA	K/RxS (100%)	K/KxS (1%)
PAK1	RxS (100%)	KxS (3%)

Tuazon, et al. Biochem 1997

WT ER: $KKNS_{305}$

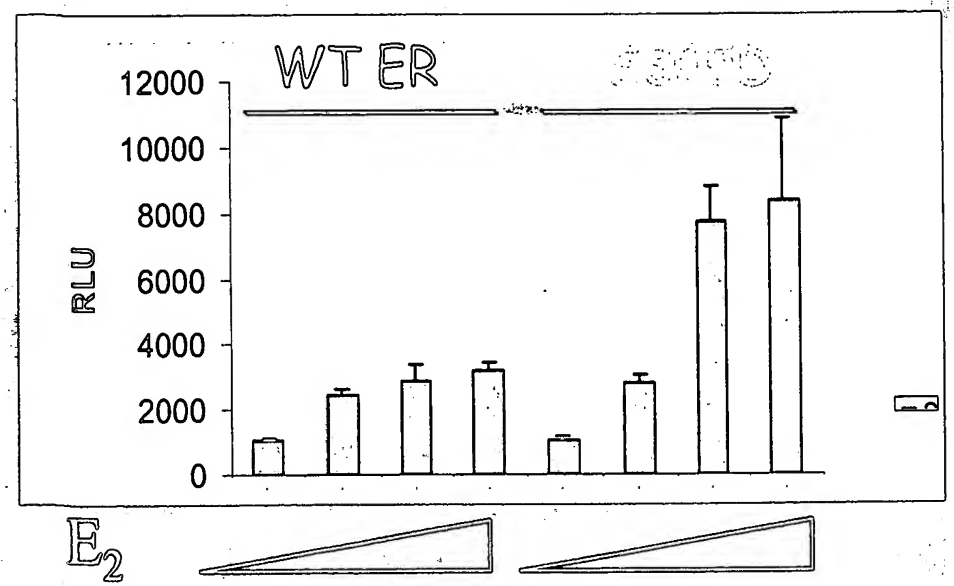
K303R: $KRNS_{305}$





ERα S305 Phosphorylation Reduces Receptor Acetylation

Transactivation Assay

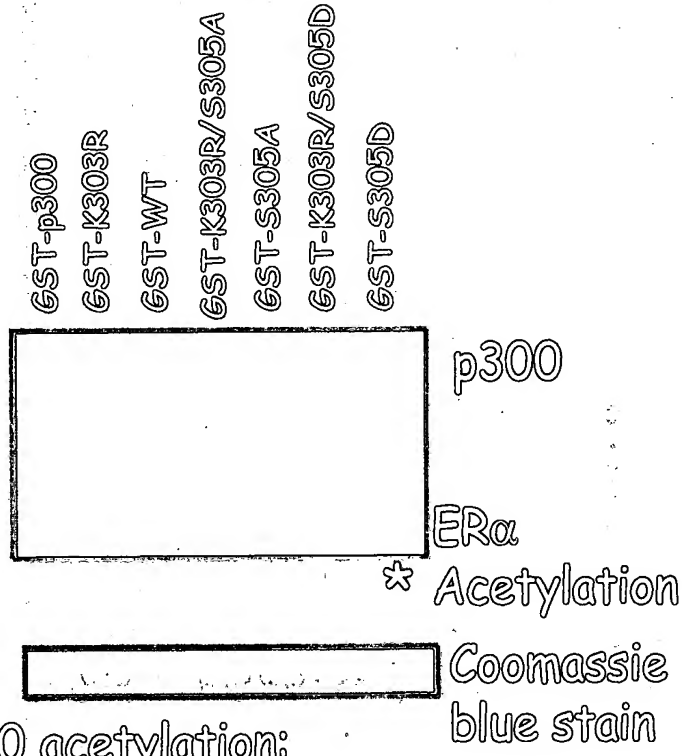


Mutant mimicking phosphorylation at S305 increases estrogen sensitivity

Acetylation Assay:
GST-ERs (Hinge #253-310)

↓

Acetylate with p300



Other examples where phosphorylation blocks p300 acetylation:
Positive co-activator PC4, Forkhead TF FOXO 3a

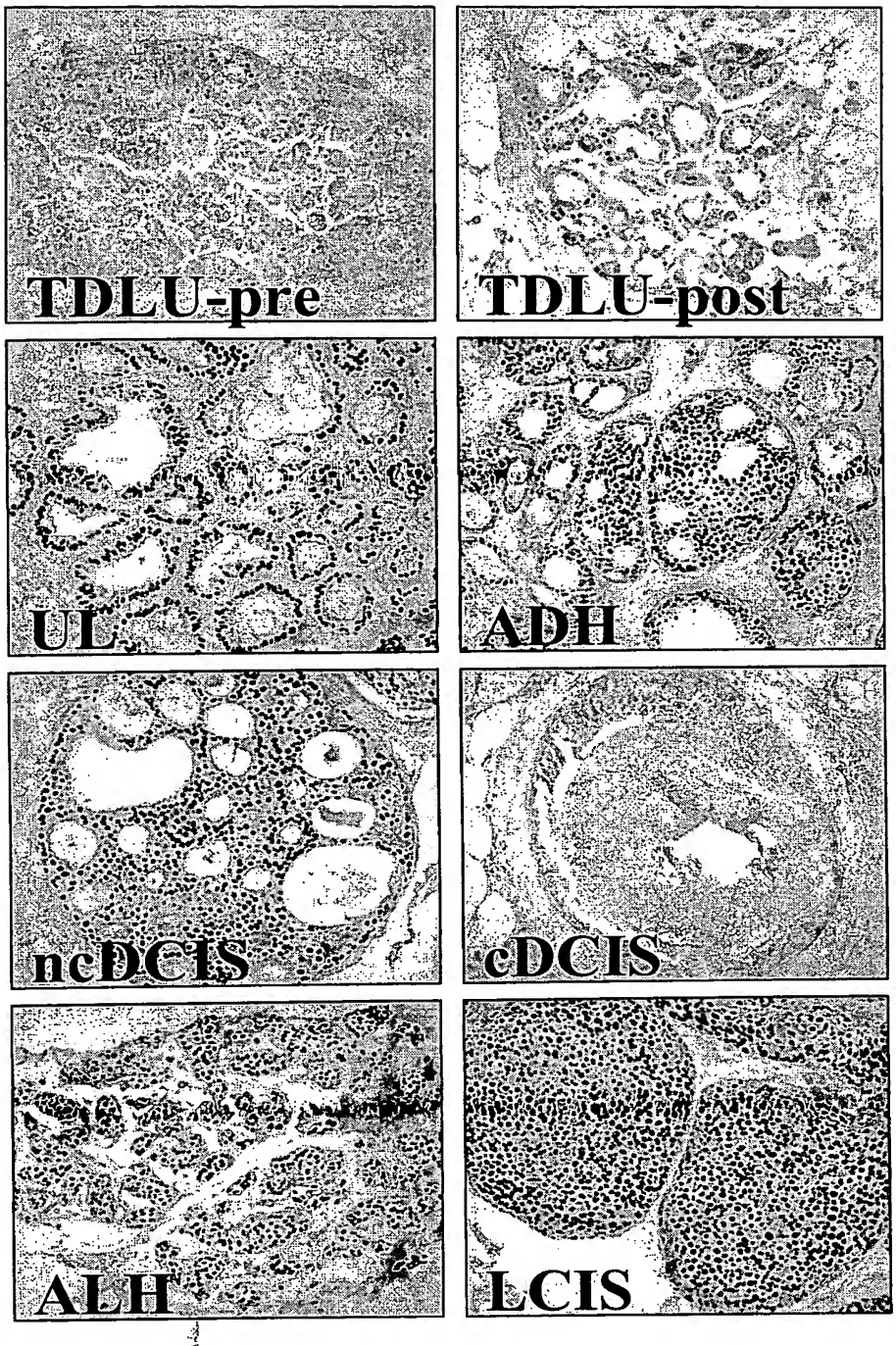


FIG. 1

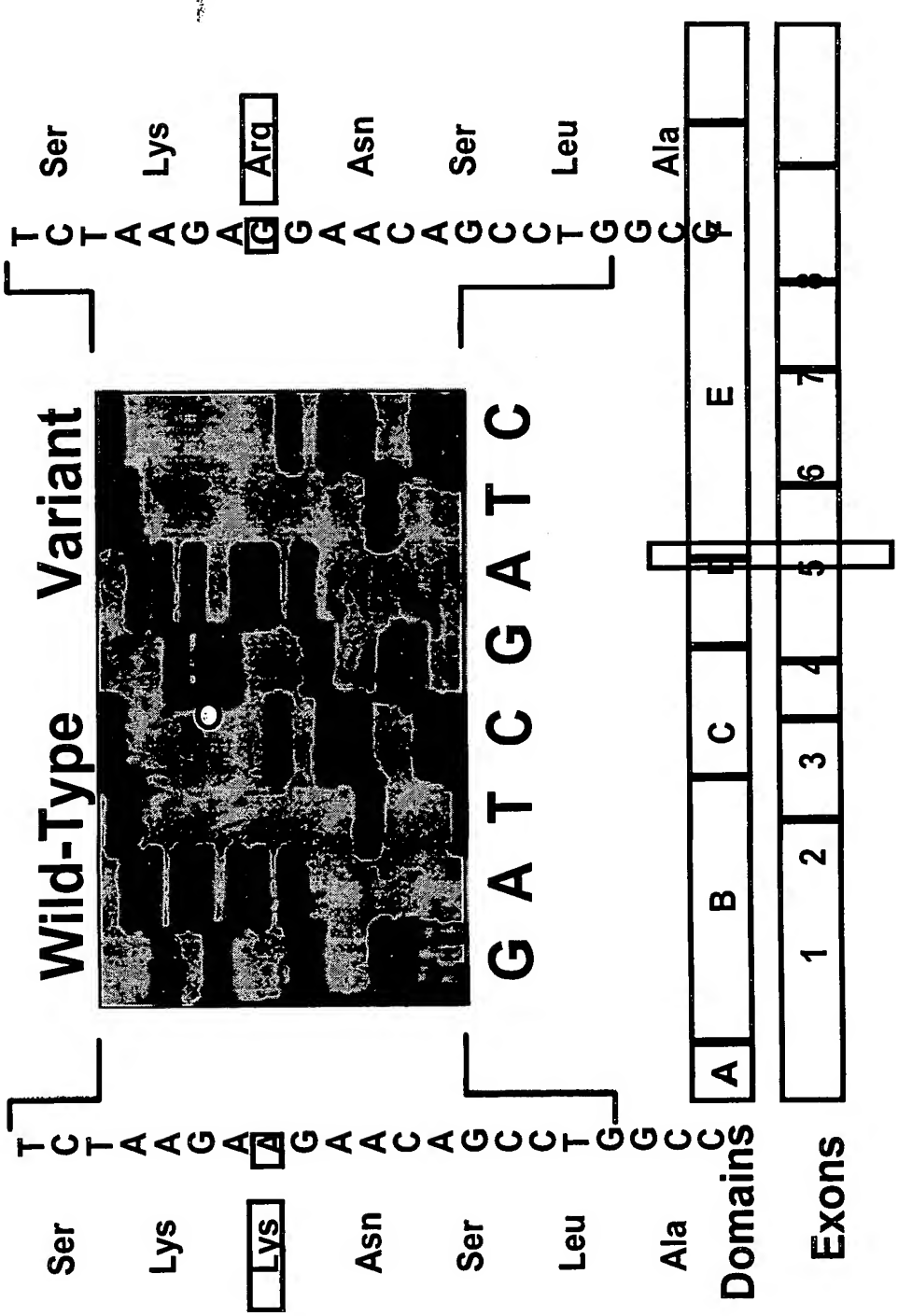


FIG. 2

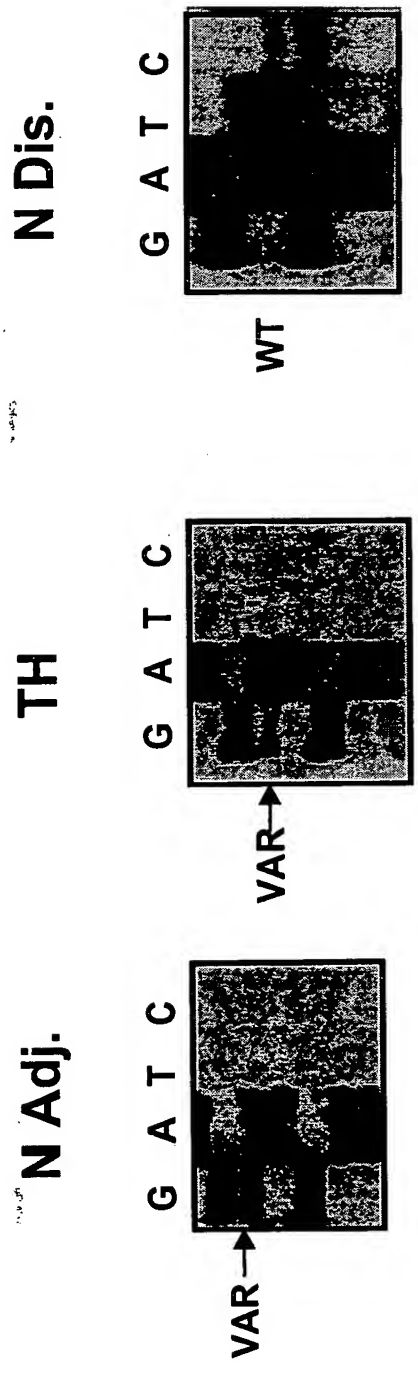


FIG. 3

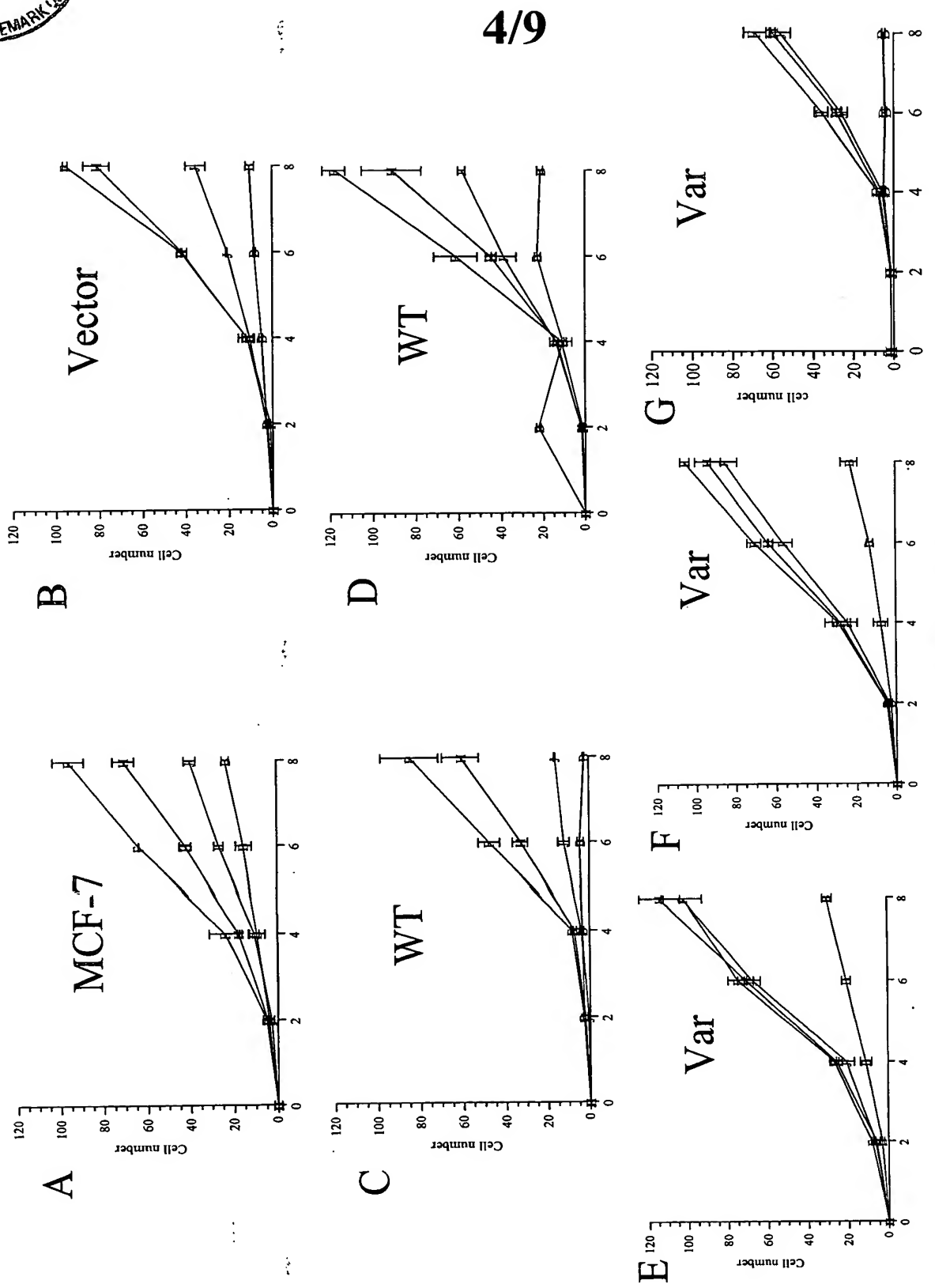


FIG. 4

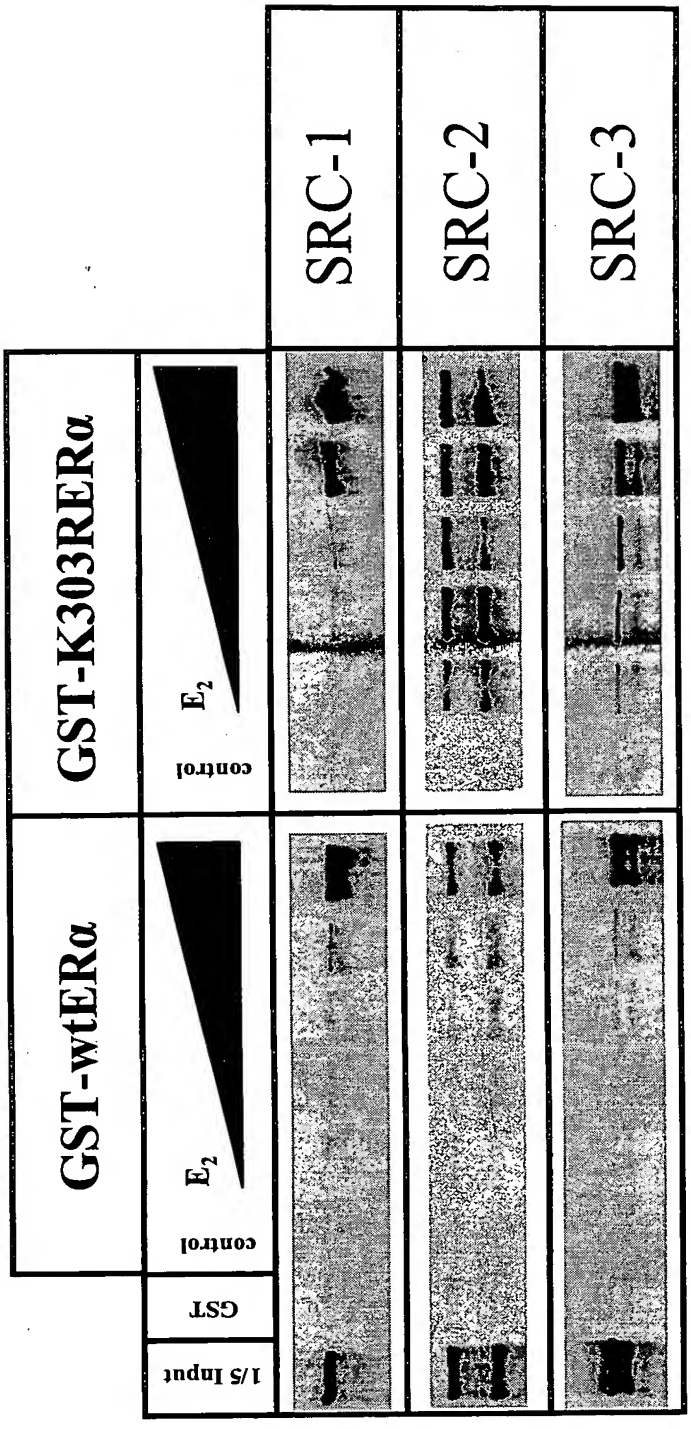


FIG. 5

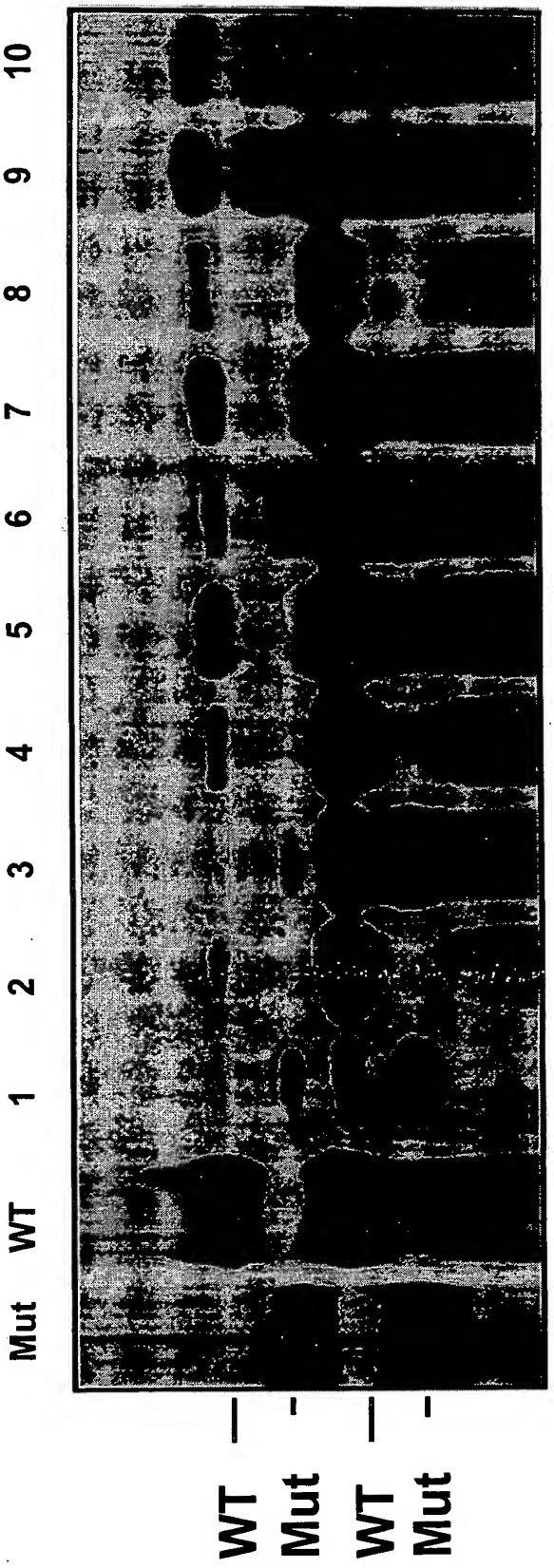


FIG. 6

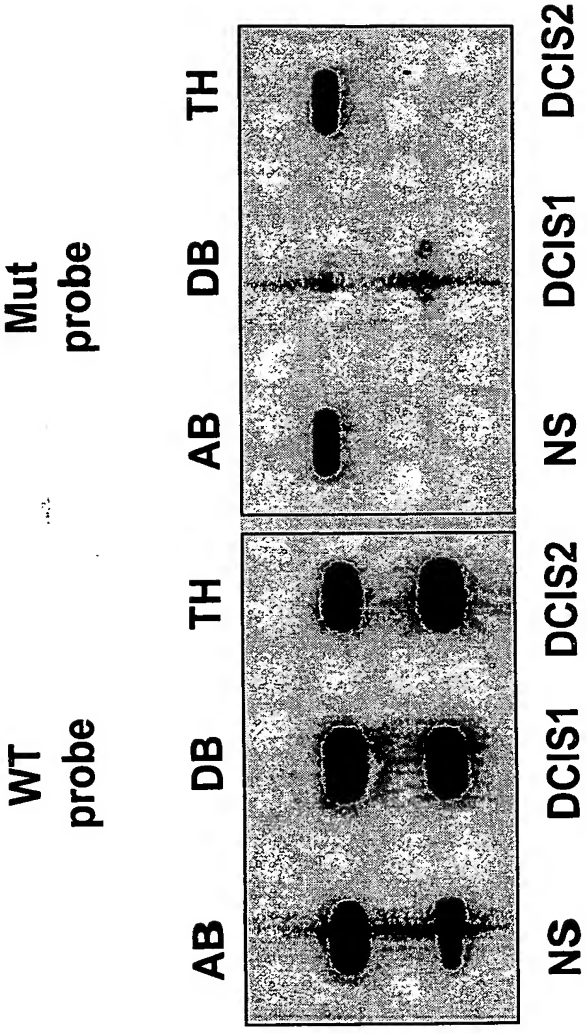


FIG. 7

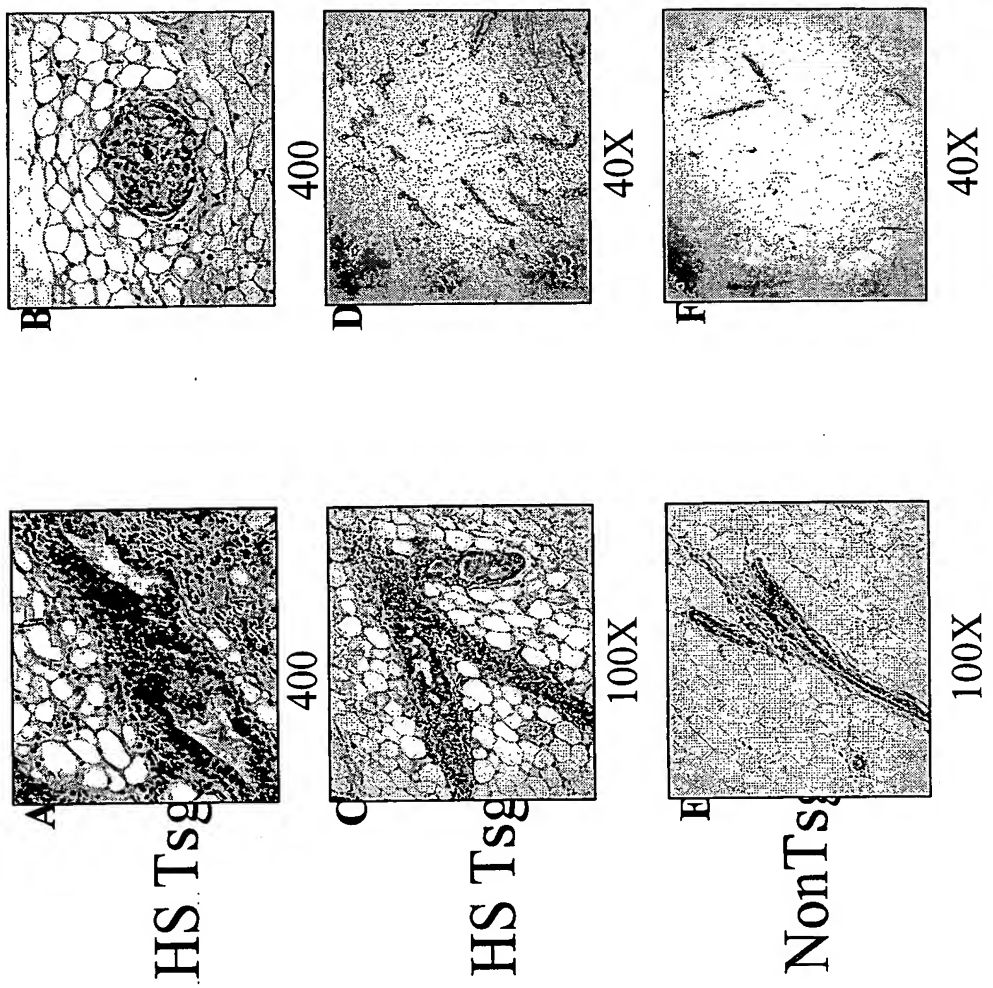
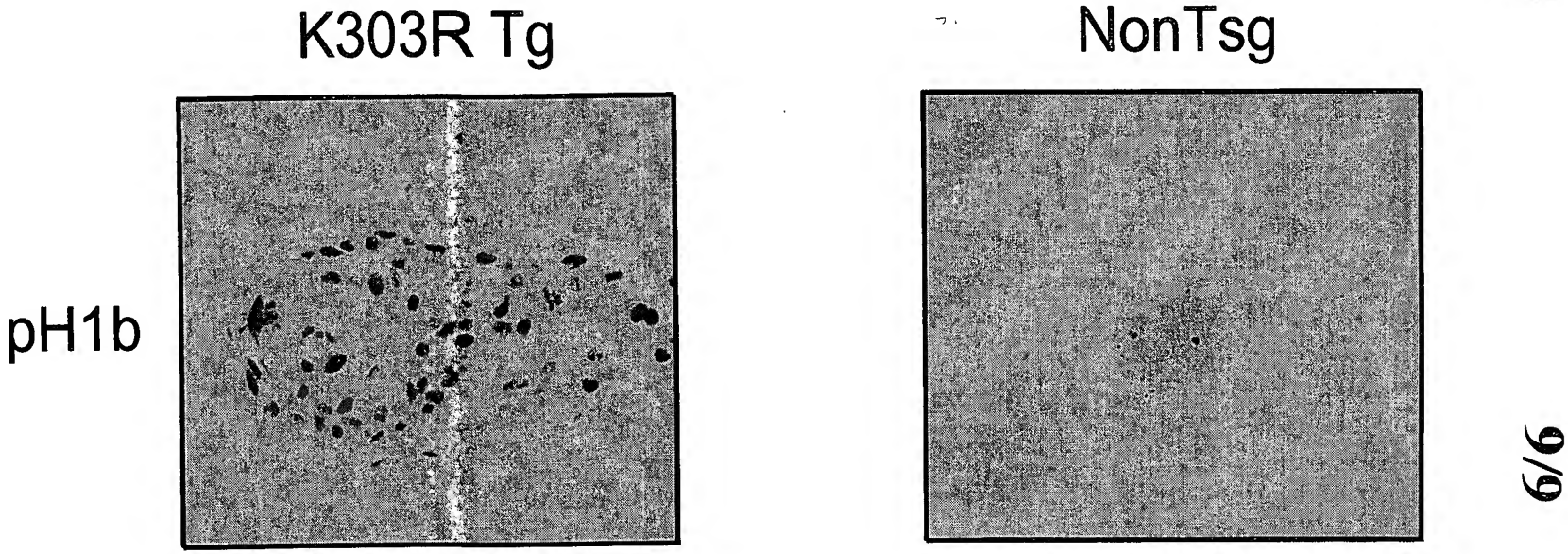


FIG. 8



18 month old virgin mice – pH1b IHC

FIG. 9